are underway. Together these experiments will allow us to define the mechanism of paladin's action and understand its role in regulating neural crest cell development. Supported by NIH F32DE019973; Minnesota Medical Foundation.

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Program/Abstract # 310
Proliferation dynamics associated with cranial neural crest cell migration

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Neural crest (NC) cells frequently divide within the neural tube prior to exiting, but also as they migrate toward their target destinations. It is still unclear whether there is a pattern to the timing and position of NC cell proliferation along the migratory route and whether this helps to promote migration. In this study, we explored the dynamics of NC cell proliferation in the r4 migratory stream in the avian embryo. We measured the effect of division on the speed of migrating cells, the timing and position of cell divisions, and whether there was a pattern to the orientation angle of division of these cells during migration. In order to analyze NC cell proliferation at the population level, we measured cell cycle and proliferative changes between different subpopulations within a cranial (r4) migratory stream using BrdU incorporation, flow cytometry and photoactivation. We observed significant differences in proliferative capabilities between cells in the leading and trailing subpopulations. We also used immunohistochemistry to assess levels of Ki67 and cleaved caspase-3 as readouts of the number of cycling and apoptotic cells, respectively, throughout the r4 migratory stream. Our data suggests that there are differences in NC cell proliferation as a result of spatiotemporal location within the stream, and that these differences contribute to the colonization of the target destinations.

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Program/Abstract # 311
Lead and trailing cranial neural crest cells display distinct cellular and molecular profiles in response to surrounding microenvironments during migration

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Neural crest (NC) cell migration is an excellent model to study how cells acquire direction and maintain a migratory stream over long distances. We previously discovered evidence for NC cell chemotaxis, as well as differences in morphology and proliferation between lead and trailing NC cells, yet it is unclear when NC cells acquire direction to the chemotactic signals and how their identity is influenced by each other and the surrounding microenvironments. By analyzing NC cell nuclear orientations, we determined that NC cells establish cellular profiles along their migratory route with lower orientation at the lead and immediately after emerging from the neural tube. We next determined that lead and trailing NC cells not only differ from one another in position within the stream, but also in gene expression. We used qPCR of FACS isolated lead and trailing NC cells to examine the expression of 84 genes and found differential expression of 43 genes. When trailing NC cells were transplanted to the lead of the NC cell migratory stream, they remained at the lead of the stream, and their gene expression profile was more similar to that of lead NC cells. When trailing NC cells were ablated, remaining lead NC cells compensated for the loss of the trailing NC cells and spread out to mimic a typical NC cell migratory stream at both the morphometric and molecular levels. Our results suggest that NC cell gene expression is dependent on NC cell-cell communication and surrounding microenivonment, not on the NC cells' origin. This analysis provides valuable insights into the mechanisms of directed NC cell migration, the behavioral and molecular differences between lead and trailing NC cells, and the plasticity of the NC cell identity.

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Program/Abstract # 312
Essential functions of the ADAM13 cytoplasmic domain in cranial neural crest cell migration

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ADAMs are transmembrane metalloproteases that control cell behavior by cleaving both cell adhesion and signaling molecules. The cytoplasmic domain of ADAMs can regulate the proteolytic activity by controlling the subcellular localization and/or the activation of the protease domain. We have recently shown that the cytoplasmic domain of ADAM13 is cleaved and translocates into the nucleus. Preventing this translocation renders the protein incapable of promoting cranial neural crest (CNC) cell migration in vivo, without affecting its proteolytic activity. In addition, the cytoplasmic domain of ADAM13 regulates the expression of multiple genes in CNC, including the protease Calpain-8. Restoring the expression of Calpain-8 is sufficient to rescue CNC migration in the absence of the ADAM13 cytoplasmic domain. Our work shows that the cytoplasmic domain of ADAM metalloproteases can perform essential functions in the nucleus of cells and may contribute substantially to the overall function of the protein.

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Program/Abstract # 313
Role of endothelin-A receptor in cardiac neural crest cell fate

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Congenital cardiovascular malformations are the most common birth defects affecting children but their cause generally remains unknown. Several of these defects occur in structures developing from neural crest cells (NCC). These NCC originate from the neural fold and migrate ventrally to populate the pharyngeal arches. During cardiovascular development, cardiac NCC (CNCC) participate in the asymmetric remodeling of the pharyngeal arch arteries into the great vessels and the septation of the outflow tract into the pulmonary and aortic outflows. One of the key signaling pathways regulating CNCC development involves Endothelin-A receptor (Ednra). The absence of Ednra signaling in the mouse causes severe cardiovascular defects, including persistent ductus arteriosus and coarctation of the aorta. However, the exact function of Ednra signaling in CNCC is unknown. We mapped the fate of CNCC in the cardiovascular system of the Ednra mouse and analyzed the survival and proliferation of these cells. Our data indicate that the migration of CNCC is aberrant in the cardiac outflow tract of the Ednra mutant embryos, but not in the pharyngeal arches. This migratory defect remains by E18.5 and appears to be independent of CNCC proliferation and apoptosis

changes at E10.5. From these results, we hypothesize that the migration of the CNCC is arrested prematurely because they fail to activate specific genes necessary for their migration and to distinguish themselves from the CNCC in the pharyngeal arch arteries. In the future, we will identify genes differentially expressed in the two subpopulations of CNCC regulated by Ednra signaling, which could explain CNCC behavior and the Ednra mouse phenotype.

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Program/Abstract # 315
Calcium transients in trunk neural crest reveal the dynamics of cell migration and aggregation during peripheral nervous system development
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Examining calcium events during neural crest (NC) migration has the potential to shed light on cell communication during migration and patterning events. However, typical analysis of calcium transients during embryogenesis has been limited to cultured cells using fluorescent indicators added to the culture media which make the cells easy to visualize but not necessarily representative of their in vivo behavior. Here we studied the spontaneous calcium transients during NC migration in vivo using a genetically encoded calcium sensor (GECI), GCaMP3 which is not toxic to the embryo like synthetic indicators but is still easily imaged. The GCaMP3 vector was electroporated into pre-migratory NC cells and calcium transients were visualized in vivo in whole chick embryos and in sagittal slice trunk explants using confocal time-lapse imaging. First we conclude trunk NC cells displayed significantly more calcium transients than cranial NC cells, especially once trunk NC cells reached the dorsal aorta and during aggregation into sympathetic ganglia (SG). Second, blocking of N-cadherin activity in trunk NC cells near the presumptive SG led to a dramatic decrease in the frequency of calcium transients. Finally, we found that calcium transients were predictive of trunk NC cells aggregating into a cluster to form an SG anlagen. Our data suggest a model in which calcium transients are correlated with trunk NC cell migration and aggregation of cells into discrete sympathetic ganglia and highlight the power of using GECI's during an embryogenic event.

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Program/Abstract # 316
Migration and transcriptional profiling of sacral neural crest derivatives in the lower urinary tract
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The neural crest (NC) gives rise to the peripheral nervous system including the innervation of the lower urogenital tract (LUT) which is essential for proper function of the bladder and genitalia. However, remarkably little is known about the molecular events that control the migration of sacral NC-derived cells that populate the LUT to produce pelvic neural elements. We utilized transgenic mice that express a Histone2BVenus (H2BVenus) reporter from Sox10 regulatory regions to visualize migration of sacral NC and capture these progenitors for analysis of gene expression in the developing bladder and pelvic ganglia. We imaged Sox10-H2BVenus embryos from E12-16 to determine migration routes and timing of NC cell entry into the LUT. H2BVenus+ progenitors form the anlagen of the pelvic ganglia by E12, enter the bladder by E13 and reach the bladder dome by E15. Using robust flow sorting methods, we isolated H2BVenus+ progenitors from LUT regions of transgenic embryos to obtain stage and sub-domain specific transcriptional profiles by microarray hybridization. To identify pathways that control development of LUT innervation, we compared the gene expression profiles of NC-derived progenitors against total embryo RNA. Subsequent comparisons of the up- and down-regulated genes were made between each stage and subdomain isolated. These simple two by two comparisons allowed identification of genes that are differentially expressed between NC progenitor populations. Understanding the factors that guide migration and differentiation of NC-derived progenitors during innervation of pelvic organs will help provide insight into increasingly prevalent LUT malformations and conditions, such as neurogenic bladder.

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Program/Abstract # 317
Ethanol exposure disrupts cell migration and cilia structure in developing embryos
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Ethanol (EtOH) exposure during early development can cause craniofacial malformations as well as neurological and behavioral disorders, known as Fetal Alcohol Syndrome (FAS). Craniofacial (CF) structures and parts of the nervous system (NS) are derived from cranial neural crest cells (CNCC) and these migratory precursors are susceptible to EtOH exposure. Premigratory CNCCs share a common border with the cells of the olfactory placode precursor (OPP) field; therefore we propose that OPPs as well as CNCC progenitor migration is disrupted by EtOH exposure. Primary cilia (PC), important for transduction of morphogens, in CNCCs are important in cell migration. Abnormalities in PC structural/functional can cause phenotypes similar to those observed in FAS. Thus EtOH exposure may disrupt PC structure/function in the CNCCs, resulting in cell migration defects. Using zebrafish as a model system, we show that EtOH can induce phenotypes similar to FAS patients; embryos show a series of craniofacial and axial malformations in a dose-dependent manner. We also show that EtOH disrupts the chemokine-signaling pathway, necessary for both olfactory placode formation and CNCC migration. Using a new quantification method, "optical flow" image processing, we show that migration of CNCC populations in developing embryos is affected by EtOH. Strikingly, we have shown that EtOH reduces the number of PC in CNCCs and disrupts ultrastructure of PC. These novel findings suggest that EtOH-induced alteration of PC in CNCCs may underlie the structural/neural defects observed in FAS as well as other environmentally induced CF and NS malformations. Grant Sponsors: FONDECYT 1071071; NIH/NICHD05820; Nucleo Milenio Mideplan, CONICYT.

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